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Synthesis and biological activity of highly potent octapeptide analogs of somatostatin

(Solid-phase peptide synthesis/structure–activity relationship/inhibition of growth hormone, insulin, glucagon, and gastric acid secretion/antitumor activity)

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ABSTRACT In the search for selective and long-acting analogs of somatostatin, nearly 200 compounds were synthesized by solid-phase methods, purified, and tested biologically. Among these octapeptides, some contained N-terminal D-Phe, Ac-D-Phe, or AcPhe followed by hexapeptide sequences Cys-Phe-D-Trp-Lys-Thr-Cys or Cys-Tyr-D-Trp-Lys-Val-Cys and Thr-NH_2 or Trp-NH_2 as C-terminal residues. D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 were 177 times and 113 times more potent, respectively, than somatostatin in tests for inhibition of growth hormone release. These two octapeptides containing tyrosine and valine in positions 3 and 6, respectively, were more active and more selective than their Phe-3 and Thr-6 counterparts, D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 and D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Trp-NH_2. D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 was also about 6 times more potent than its L-Trp-4 diastereoisomer. The analogs D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 showed a prolonged duration of action and were able to inhibit growth hormone release for at least 3 hr. Analogs of both Phe-3/Thr-6 and Tyr-3/Val-6 classes also suppressed the release of insulin and glucagon in rats and pentagastrin-induced secretion of gastric acid in dogs, but their potencies in these tests were much smaller than the growth-hormone-release inhibitory activity. Some of these analogs possessed antitumor activities as shown by the inhibition of growth of animal models of prostate, mammary, and ductal pancreatic tumors.

The tetradecapeptide somatostatin (also called somatostatin-14) is of little therapeutic value since it has a broad spectrum of biological actions and a short half-life (1). Conformational analyses and structure–function studies on somatostatin analogs indicate that the sequence required for biological activity consists of the β-turn fragment Phe-Trp-Lys-Thr corresponding to the residues 7–10 of somatostatin (2, 3). Many somatostatin analogs with smaller and more rigid rings have been designed and synthesized in the search for compounds with selective, enhanced, and prolonged activity (2–6). Veber and co-workers (3, 4) reported that cyclic hexapeptide analogs cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe) and cyclo(*N*-MeAla-Phe-D-Trp-Lys-Thr-Phe) were highly active in tests on the inhibition of growth hormone (GH), insulin, and glucagon release. More recently, the same group synthesized a new hexapeptide, cyclo(*N*-MeAla-Tyr-D-Trp-Lys-Val-Phe), containing tyrosine and valine in positions corresponding to the residues 7 and 10, respectively, of somatostatin, and found it to be 50–100 times more potent than the parent molecule (5).

Bauer *et al.* (6) synthesized another series of highly potent octapeptide analogs of somatostatin. They retained the sequence of residues 7–10 of somatostatin, Phe-Trp-Lys-Thr, and incorporated this sequence with the tryptophan residue in the D configuration into a series of cystine-bridged analogs of which D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol) (code no. SMS 201-995) containing a C-terminal amino alcohol was the most active (6). This analog has been subjected to careful clinical evaluation (7, 8).

Recently, nearly 200 octapeptide amide analogs of somatostatin related to compound SMS 201-995 made by Bauer *et al.* (6) have been synthesized by solid-phase methods in our laboratory (9, 10). This paper reports the synthesis and the evaluation of biological activities of some of these analogs.

MATERIALS AND METHODS

Synthesis. The analogs were synthesized in a Beckman model 990 automatic peptide synthesizer using standard solid-phase procedures (11). Benzhydrylamine resin (0.50 mmol/g) was used as starting material. Amino acids were coupled as their N^{α}-*tert*-butoxycarbonyl (Boc) derivatives, and reactive side chains were protected as follows: serine and threonine, with benzyl; cysteine, with 4-methylbenzyl; lysine, with 2-chlorobenzyloxycarbonyl; tyrosine, with 2-bromobenzyloxycarbonyl. After the incorporation of D- or L-tryptophan, 2-mercaptoethanol was added to the CF_3COOH/CH_2Cl_2 solution for all subsequent deblocking steps. The octapeptide amides were cleaved from the resin with simultaneous deprotection using liquid HF. The disulfhydryl peptides were cyclized either by the usual potassium ferricyanide method (12) or by using iodine (13).

Analytical HPLC. The purity of the final products was established in two different HPLC systems; on a C_{18} column (VYDAC 218TP546) eluted with solvents A (0.1% aqueous CF_3COOH) and B (0.1% CF_3COOH in 70% CH_3CN) (system I) or on a C_4 column (VYDAC 214TP546) eluted with solvent A (0.02 M CH_3COONH_4, pH 4.1) and solvent B (70% CH_3CN/30% solvent A, vol/vol) (system II).

Purification. After cyclization, disulfide peptides were subjected to gel filtration on a 2.5 × 70 cm Sephadex G-15 column in 30% AcOH. Pooled fractions of gel filtration were further purified by semipreparative HPLC on a 10 × 250 mm VYDAC C_{18} column or on a 21.4 × 250 mm DYNAMAX C_{18} (Rainin Instruments, Woburn, MA) cartridge. The peptides were eluted isocratically or by a flat gradient (0.1–0.2%

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Abbreviations: GH, growth hormone; b.i.d., twice a day.

solvent B per min) using the solvent system containing 0.1% CF_3COOH. Crude disulfhydryl peptides were purified in a similar way, except that no gel filtration was used.

Amino Acid Analyses. Amino acid analyses were performed in a Beckman 119 amino acid analyzer on samples that were hydrolyzed (110°C for 18 hr) in 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole in sealed, evacuated tubes.

Biological Assays. Adult Sprague–Dawley male rats weighing 200–350 g were used in all bioassays.

GH Potency Assay. The rats were anesthetized with sodium pentobarbital (60 mg/kg of body weight, administered i.p.). Thirty minutes later the somatostatin analogs or saline were injected s.c., and blood samples were drawn from the jugular vein 15 min after injection. The plasma was separated and assayed for GH by RIA (14). The potencies were calculated by four-point assay (15) and expressed as the percentage of somatostatin activity.

GH Time-Course Assay. The rats were treated with sodium pentobarbital as in the GH potency assay or with thiamylal (Surital) (5 mg/100 g of body weight, administered i.p.) and with morphine (2 mg/rat, administered s.c.) and injected with the somatostatin analogs or saline. Sodium pentobarbital or thiamylal at half the initial dosage was given at 60- to 90-min intervals to maintain anesthesia. Blood was collected from the jugular vein 15, 30, 60, 120, 180, and 240 min after the injection of analogs.

Insulin and Glucagon Assays. Rats were fasted for 27–30 hr to raise plasma glucagon levels and then were anesthetized with sodium pentobarbital. After 30 min, the somatostatin analogs or saline were injected into the jugular vein, and 5 min later blood was collected from the hepatic portal vein. Plasma was assayed for insulin and glucagon by RIA (5, 6, 9, 16).

Inhibition of Gastric Acid Secretion. Mongrel dogs weighing 15–20 kg were prepared surgically with gastric fistulae (17) and fasted overnight. Gastric acid secretion was stimulated by infusion of pentagastrin or desglugastrin (3 μg/kg per hr). The analogs of somatostatin were infused intravenously. Gastric acid was collected, and the total acidity was determined by titration with 0.1 M NaOH. Inhibitory responses obtained with the analogs at two-dose levels were compared to those induced by somatostatin and calculated by factoral analyses (15).

Antitumor activities in animal models of prostate, mammary, and ductal pancreatic cancers were measured as described (9, 18). The methods used for following inhibitory effects on osteosarcomas and pituitary tumors were also reported (9, 19).

RESULTS

Octapeptide amides related to D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol) (6) containing Phe-3 and Thr-6 or Tyr-3 and Val-6 and modified at the *N*- and C-terminal residues were synthesized by solid-phase methods, purified, and tested to evaluate the effect of these substitutions on the biological activity. The crude products, both disulfhydryl peptides cleaved from the resin and disulfide peptides obtained after oxidation, were analyzed by HPLC and showed good quality. The oxidation of disulfhydryl octapeptides was generally performed with potassium ferricyanide. However, the oxidation of peptides containing two tryptophan residues was carried out with iodine because of their poor solubility under the conditions used for oxidation with potassium ferricyanide. This change in the oxidation method increased the yield from 4–7% to 25% (Table 1). Gel filtration of these highly hydrophobic analogs was accomplished with dual elution: first with water, followed by 50% AcOH. This prevented the coelution of salts and peptides that normally would have occurred because of adsorptive interactions between the aromatic side chains of the analogs and the gel.

After purification, the homogeneity of peptides was examined by analytical HPLC in two different solvent systems. The peptides were eluted as single symmetrical peaks, and in all cases the purity was found to be greater than 95% based on UV absorbance at 214 nm. The purified octapeptides were also characterized by amino acid analyses of acid hydrolysates, which gave the expected composition. Analytical data for the purified products are given in Table 1.

When the biological activity on inhibition of GH release was measured *in vivo* in sodium pentobarbital-anesthetized rats, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (code no. RC-121) with Tyr-3 and Val-6 was found to be 177 times more potent than somatostatin (Table 2). This analog was also about 4 times more active in tests for inhibition of GH release than the corresponding peptide with Phe-3 and Thr-6, D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-102), and approximately 6 times more potent than D-Phe-Cys-Tyr-L-Trp-Lys-Val-Cys-Thr-NH_2 (RC-159-II), which has Trp-4 in the L configuration. Ac-*p*-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-15), was only about 10 times more potent than somatostatin. In the same GH inhibition assay, the activity of the analog D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160) with Tyr-3 and Val-6 was 113 times higher than that of somatostatin and 2 times greater than that of D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Trp-NH_2 (RC-95-I), which con-

Table 1. Characterization and yields of somatostatin analogs

Structures	Code no.	Amino acid analyses, no. of residues								Purity,* %	Yield,† %
		Phe	Cys	Tyr	Trp	Lys	Thr	Val	NH_2		
D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2	RC-102	2.00	2.12		0.96	0.98	2.08		1.19	97	26
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	RC-121	1.00	1.91	1.00	0.84	0.95	1.08	1.08	1.16	98	25
D-Phe-Cys-Tyr-L-Trp-Lys-Val-Cys-Thr-NH_2	RC-159-II	1.00	1.09	1.00	0.87	0.94	1.09	1.00	1.20	96	22
D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Trp-NH_2	RC-95-I	2.00	1.94		1.91	0.96	1.04		1.18	95	4§
D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2	RC-160	1.00	1.92	0.99	1.90	1.06		1.01	1.04	97	7§, 25‡

*Based on the integration results of analytical HPLC runs monitored at 214 nm.
†Overall yield based on the molar equivalent of starting benzhydrylamine resin.
‡Oxidation of disulfhydryl peptide was carried out with potassium ferricyanide.
§Oxidation of disulfhydryl peptide was carried out with iodine.

tained Phe-3 and Thr-6. Both analogs DPhe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and DPhe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 showed significant inhibition of GH release at doses as low as 0.005–0.02 μg/100 g of body weight. In time-course studies, a prolonged inhibition of GH release for a period of at least 3 hr was observed for both of these analogs at a dose of 0.1 μg/100 g of body weight in contrast to a short duration of action of 2 μg/100 g of body weight of somatostatin (Fig. 1). This demonstrates that these analogs are long-acting in addition to being superactive. An attempt was also made to calculate the potency of the analogs by using integrated GH levels as a parameter of GH-inhibitory activity. Mean serum GH levels were plotted on an arithmetic graph against time, and the areas under the curves of the three respective groups, as compared with the saline-injected group, were calculated. These areas were arbitrarily considered as the integrated serum GH levels during the period after the injection. The areas indicating the depression of the integrated serum GH levels for analogs RC-121 and RC-160 were 158 times and 134 times greater, respectively, than that of somatostatin, after allowing for the difference in doses. The structures of other typical somatostatin analogs synthesized and their inhibitory activity on GH release are shown in Table 3.

The inhibitory activities of RC-102, RC-95-I, RC-121, and RC-160 on *in vivo* release of insulin were 38, 34.5, 8.7, and 6.2 times greater, respectively, than that of somatostatin (Table 4). Since analogs RC-121 and RC-160 with Tyr-3 and Val-6 had much higher potency for inhibition of GH secretion but lower activity for inhibition of insulin release than did analogs RC-102 and RC-95-I with Phe-3 and Thr-6, this suggests that the Tyr-3/Val-6-containing series of analogs might be more specific for GH inhibition. The inhibition of glucagon release by analogs RC-121 and RC-160 was greater than that for insulin (Table 4). The gastric acid-inhibitory potencies of analogs D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 were 4.75 and 4.33 times greater, respectively, than that of somatostatin (Table 4). Moreover, the inhibition of gastric acid secretion appeared to be protracted in contrast to somatostatin, whose action is short-lived.

Table 2. Relative potencies of somatostatin analogs in inhibition of GH release *in vivo* based on four-point assays

Code no.	Dose, μg/100 g BW	Plasma GH,* ng/ml	Potency,† %
Saline		427 ± 226	
SS-14	0.4	211 ± 89	100
	1.6	106 ± 24	
RC-102	0.02	143 ± 44	4500
	0.08	69 ± 2	(760–26880)
Saline		264 ± 69	
SS-14	0.4	74 ± 14	100
	1.6	46 ± 7	
RC-121	0.005	55 ± 5	17650
	0.02	42 ± 6	(4460–69800)
RC-159-II	0.02	64 ± 12	2795
	0.08	44 ± 10	(732–10680)
Saline		157 ± 57	
SS-14	0.4	65 ± 10	100
	1.6	45 ± 7	
RC-95-I	0.02	51 ± 7	5280
	0.08	31 ± 4	(2065–13580)
Saline		364 ± 136	
SS-14	0.4	160 ± 29	100
	1.6	74 ± 4	
RC-160	0.02	53 ± 8	11330
	0.08	37 ± 9	(3264–39300)

SS-14, somatostatin-14; BW, body weight.
*Mean ± SEM (five to six rats per group).
†Potency was determined by four-point assay. The 95% confidence limits are given in parentheses.

In tests in animal tumor models, various analogs of both the Phe-3/Thr-6 and Tyr-3/Val-6 series were shown to possess significant antitumor activity. Analog Ac-*p*-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-15) in doses of 3 μg twice a day (b.i.d.), inhibited the growth of estrogen- and

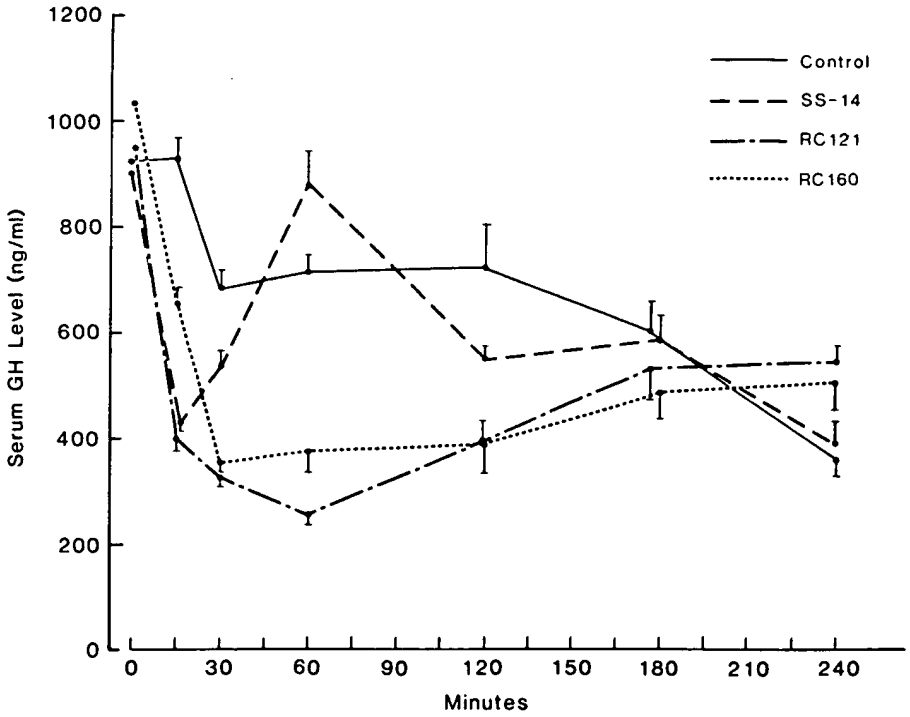

FIG. 1. Time course of inhibition of thiamylal/morphine-stimulated GH secretion in rats by somatostatin-14 and its analogs. The analogs RC-121 and RC-160 were injected s.c. at a dose of 0.1 μg/100 g of body weight and somatostatin-14 was injected s.c. at 2 μg/100 g of body weight at time zero. Values shown are means ± SEM.

prolactin-dependent MT/W9A mammary adenocarcinoma in Wistar/Furth rats (9). In Dunning R3327H model of prostate cancer in Copenhagen–Fisher rats, administration of 2.5 μg b.i.d. of analog RC-121 resulted in a decrease in tumor growth. Both analogs, RC-121 and RC-160 in doses of 2.5 μg b.i.d. inhibited the growth of well-differentiated ductal pancreatic tumors in golden hamsters in agreement with results obtained earlier with less potent analogs of somatostatin (9, 20).

The analogs Ac-*p*-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-15) and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160-2H) in doses of 2–5 μg b.i.d. appeared to have antitumor activities as shown by a more than 50% increase in survival rate in mice bearing the Dunn osteosarcoma in short-term studies (9). All of these studies will be reported in detail elsewhere.

DISCUSSION

D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol) (code no. SMS 201-995) is a potent long-acting octapeptide analog of somatostatin with C-terminal amino alcohol (6). Our somatostatin analogs reported in this paper are octapeptides related to the compound of Bauer *et al.* (6) but contain C-terminal amide that is present in many natural peptide hormones and can be synthesized by standard solid-phase methods.

The cyclic hexapeptide cyclo(*N*-MeAla-Tyr-D-Trp-Lys-Val-Phe), in which tyrosine and valine replaced phenylalanine and threonine in positions corresponding to residues 7 and 10, respectively, of somatostatin, was found to be about 15 times more potent than cyclo(*N*-MeAla-Phe-D-Trp-Lys-Thr-Phe) (5). Consequently, we felt that it would be worthwhile investigating the effect of these substitutions—i.e., the replacement of phenylalanine and threonine by tyrosine and valine in positions 3 and 6, respectively, of the octapeptide sequence—on the potency of our analogs. However, we found that the activity of the resulting analogs is influenced not only by these substitutions but also by the nature of the residues of the N and C terminus of the octapeptides (Table 3). Analogs D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (RC-121) and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160) with C-terminal Thr-NH_2 and Trp-NH_2 show an enhanced potency with respect to the corresponding analogs D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-102) and D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Trp-NH_2 (RC-95-I). In contrast to this, the same substitutions in the octapeptide D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Pro-NH_2 (RC-76-2H) with C-terminal Pro-NH_2 resulted in a decrease in activity by a factor of 10 (Table 3). In analogs with other C-terminal amino acid residues, the substitutions of tyrosine and valine in positions 3 and 6 do not cause in most cases significant differences in potency (10). The importance of the side-chain hydroxyl group in the C-terminal residue is indicated by the

Table 4. Relative *in vivo* potencies of somatostatin analogs on inhibition of insulin and glucagon release in rats and gastric acid secretion in dogs

Code no.	% inhibition		
	Insulin	Glucagon	Gastric acid
SS-14	100	100	100
RC-102	3800 (1300–10400)	—	—
RC-121	875 (162–4663)	1920 (426–8662)	475 (403–559)
RC-95-I	3450 (123–96900)	—	—
RC-160	620 (253–1502)	970 (240–3916)	433 (371–505)

There were five to six rats per group. Potency was determined by four-point assay. The 95% confidence limits are given in parentheses. SS-14, somatostatin-14.

Table 3. Structures and GH inhibitory activities *in vivo* of other somatostatin analogs synthesized in our laboratory

Code no.	Structure	GH inhibition, %
SS-14	Somatostatin-14	100
RC-115-II-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Gly-NH_2	95
RC-138-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Ala-NH_2	1570
RC-114-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Ser-NH_2	4280
RC-150-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Abu-NH_2	1530
RC-121-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	11800
RC-164-I-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Phe-NH_2	2400
RC-157-2H	D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Tyr-NH_2	4220
RC-113-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Tyr-NH_2	3020
RC-160-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2	5540
RC-76-2H	D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Pro-NH_2	1070
RC-161-2H	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Pro-NH_2	95
RC-101-I	AcPhe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2	7060
RC-161	Ac-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	5520
RC-102-II	AcPhe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	4600
RC-88	*p*-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2	2130
RC-88-II	*p*-Cl-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	1740
RC-15	Ac-*p*-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2	860

Abu, 2-aminobutyric acid.

finding that the octapeptides containing Ser-NH_2 and Thr-NH_2 at the C-terminus are more potent than the respective Ala-NH_2 and Abu-NH_2 (Abu, 2-aminobutyric acid) analogs (Table 3). However, the phenolic hydroxyl group in the ^{8}Tyr-NH_2 octapeptide (RC-113-2H) did not result in an increase in potency as compared to the ^{8}Phe-NH_2 analog (RC-164-I-2H) (Table 3).

It was hypothesized by Bauer *et al.* (6) from conformational studies that the presence of D-Phe-1 and Thr(ol)-8 is required to stabilize the active conformation. In a recent study (21), it indeed was found that the β-turn/β-sheet conformation is stabilized by the N- and C-terminal amino acids D-Phe-1 and Thr(ol)-8 through intramolecular hydrogen bonds. The importance of intramolecular hydrogen bonds can be demonstrated by the low potency of our analog with the C-terminal Pro-NH_2 residue, RC-116-2H. The Pro-NH_2 at the C terminus prevents the formation of an intramolecular hydrogen bond. High potency of analogs with Trp-NH_2 (RC-160 and RC-95-I) may be due to two factors stabilizing their biologically active conformation. One could be the formation of the intramolecular hydrogen bond between the N- and C-terminal peptide bonds. In addition, C-terminal tryptophan could be involved in the internal stabilization of the tertiary structure of these analogs through the stacking of aromatic rings. Alternately, Trp-8 may increase receptor affinity. This could explain why the C-terminal threonine residue, normally thought of as hydrophilic, can be replaced with a hydrophobic residue such as tryptophan without major loss in activity.

We observed that octapeptide analog RC-121 with Trp-4 in the D configuration is more potent than its L-Trp-4 diastereoisomer (RC-159-II). This is in agreement with the results recorded for tetradecapeptide analogs of somatostatin (22, 23). However, there is no significant difference in the biological activity between D- and L-tryptophan diastereisomers of most of the highly rigid cyclic hexapeptide analogs (24). Conformational studies indicated that the analog SMS 201-995 (6) is more rigid than somatostatin but is still rather flexible in water (21). It appears that the activity-increasing effect of the D-tryptophan substitution in this position can be manifested only in flexible peptides, where some adaptation with respect to receptor binding is possible.

Both analogs RC-121 and RC-160 show a high potency and a long duration of action for inhibition of GH release and much lower activity for suppression of insulin, glucagon, and gastric acid secretion. This indicates a marked selectivity in their biological actions. These analogs could be useful for the treatment of endocrine-related diseases such as diabetes mellitus type I and diabetic retinopathy. They may also find various applications in the field of hormone-sensitive tumors. We showed that various analogs of both the Phe-3/Thr-6 and Tyr-3/Val-6 series reported herein had significant antitumor activity as evidenced by their inhibition of the growth of animal models of prostatic, breast, and pancreatic cancers (9). The clinical efficacy of these compounds remains to be demonstrated. However, theoretical considerations and collective data from the animal tumor models suggest that a new approach based on some analogs of this type could become a useful addition to the present methods of treatment of certain endocrine-dependent or hormone-sensitive tumors (9, 18, 20, 25).

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